

SUPPORTING INFORMATION

Triple-Indicator-Based Multidimensional Colorimetric Sensing Platform for Heavy Metal Ions Detections

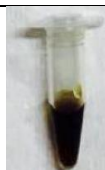
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Table S-1: Color matrix of multiple metal-ligand reactions.

Heavy metal ions/ligands	 Cu ²⁺	 Fe ³⁺	 Cr ²⁺	 Ni ²⁺	 Hg ²⁺	 Pb ²⁺
 TCP						
 -NH ₂						
 MePh				-	-	-
 12P	-	-	-		-	-
 NaI	-	-	-	-		-
 KI	-	-	-	-	-	

1. COLOR CALIBRATION ALGORITHM

The heavy metal ions (HMIs) images were captured in an RGB colour space, i.e., the device-dependent colour space. Then, the colour calibration algorithm was applied to these images, and they were finally rendered in the target colour space, i.e., the device independent sRGB colour space. To compute the colour differences, it was necessary to connect the sRGB colour space to the CIE standard colour space. The transformation of sRGB colour space to CIE XYZ colour space is defined as:

$$\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} = \Phi_{sRGB \rightarrow XYZ} \begin{pmatrix} R \\ G \\ B \end{pmatrix} = \begin{pmatrix} 0.4125 & 0.3576 & 0.1804 \\ 0.2127 & 0.7152 & 0.0722 \\ 0.0193 & 0.1192 & 0.9503 \end{pmatrix} \cdot \begin{pmatrix} R \\ G \\ B \end{pmatrix} \quad (S1)$$

The colour difference between two images is defined on the CIE $L^*a^*b^*$ colour space. This colour space is defined in the following, and the X_0, Y_0, Z_0 are the CIE XYZ values of the reference white point:

$$\begin{aligned} L^* &= 116f\left(\frac{Y}{Y_0}\right) - 16 \\ a^* &= 500 \left[f\left(\frac{X}{X_0}\right) - f\left(\frac{Y}{Y_0}\right) \right] \\ b^* &= 200 \left[f\left(\frac{Y}{Y_0}\right) - f\left(\frac{Z}{Z_0}\right) \right] \\ f(x) &= \begin{cases} x^{1/3}, & x > 0.00886 \\ 7.787x + \frac{16}{116}, & x \leq 0.00886 \end{cases} \end{aligned}$$

Finally, the Euclidean distance between two colours (L^*_1, a^*_1, b^*_1) and (L^*_2, a^*_2, b^*_2) , denoted as ΔE^*_{ab} which is more or less proportional to their colour difference as perceived by a human observer, is defined as the colour chromatic aberration between images.¹ The formula is:

$$\Delta E^*_{ab} = \sqrt{\left(\frac{\Delta L}{K_L S_L}\right)^2 + \left(\frac{\Delta C}{K_C S_C}\right)^2 + \left(\frac{\Delta H}{K_H S_H}\right)^2} \quad (S2)$$

where

$$\Delta L = L_1 - L_2$$

$$\begin{aligned}
\Delta C &= C_1 - C_2 \\
\Delta H &= \sqrt{\Delta a^2 + \Delta b^2 - \Delta C^2} \\
C_1 &= \sqrt{a_1^2 + b_1^2}, C_2 = \sqrt{a_2^2 + b_2^2} \\
\Delta a &= a_1 - a_2, \Delta b = b_1 - b_2 \\
S_L &= 1, S_C = 1 + K_1 C_1, S_H = 1 + K_2 C_1 \\
K_L = f(x) &= \begin{cases} 1, & \text{default} \\ 2, & \text{textiles} \end{cases}, K_C = 1 (\text{default}), K_H = 1 (\text{default}) \\
K_1 &= \begin{cases} 0.045, & \text{graphic arts} \\ 0.048, & \text{textiles} \end{cases}, K_2 = \begin{cases} 0.015, & \text{graphic arts} \\ 0.014, & \text{textiles} \end{cases}
\end{aligned}$$

ΔL , ΔC , and ΔH are the difference in lightness, chroma, and hue.

2. DEFINITION OF THE ALGORITHM

The principle of the colour calibration method includes: (i) using a polynomial algorithm, (ii) the corresponding quantized colour values of each colorchecker patch produced by the digital camera is represented as a vector, V : (R_k, G_k, B_k) ($k = 1, 2, \dots, 24$), and (iii) the corresponding device-independent sRGB tristimulus values are S : (SR_k, SG_k, SB_k) ($k = 1, 2, \dots, 24$).

A simple linear transform involves only R , G , and B values to map from its RGB to sRGB values. A higher order polynomial transforms more terms such as R^2, G^2, B^2 can be added to the polynomial transform, for the mapping matrix to increase the transformation accuracy. For example, if we use the combination of polynomial x : [$R, G, B, 1$], the transformation model can be represented as:

$$\begin{cases} SR_i = a_{11}R_i + a_{12}G_i + a_{13}B_i + a_{14} \\ SG_i = a_{21}R_i + a_{22}G_i + a_{23}B_i + a_{24} \\ SB_i = a_{31}R_i + a_{32}G_i + a_{33}B_i + a_{34} \end{cases} \quad (S3)$$

where $i = 1, 2, \dots, 24$

This equation also can be rewritten in the matrix format as:

$$S = A^T \cdot X \quad (S4)$$

where A is the mapping coefficient matrix and X is the matrix generated by different polynomial combinations, x . Using the least-square regression method, the solution to the Equation (S4) is as follows:

$$A = (X^T X)^{-1} X^T S \quad (S5)$$

Thus, the mapping coefficient matrix, A, can be derived to calibrate the colour tristimulus values from the device-dependent RGB colour space to the sRGB colour space. The transform can be applied to the HMI image. Suppose the polynomial image matrix X for HMI image is X_{in} , then the output image matrix X_{out} is:

$$X_{out} = A^T \cdot X_{in} \quad (S6)$$

Different combinations of polynomial terms can be used for colour calibration, as in the following combinations, where there are 4 elements in the first row, and 10 elements in the second.

$$\begin{aligned} x &: [R, G, B, 1] \\ x &: [R, G, B, R^2, G^2, B^2, RG, RB, GB, 1] \end{aligned} \quad (S7)$$

In the proposed colour calibration scheme, linear and non-linear algorithms, including first and second order polynomials, respectively, as shown in Equation (S7) were tested. The colour calibration was carried out by 3x4 and 3x10 matrices.

3. EVALUATION OF THE CALIBRATION ALGORITHM

To evaluate the performance of the calibration algorithm, the colour differences among the colour tristimulus values for each patch on the Munsell colorchecker were calculated before and after colour calibration, following Equation (S2). The total colour difference between the target and calibrated value for the whole colorchecker is calculated as

$$RMS = \sqrt{\frac{\sum_{i=1}^M \Delta E^*_i{}^2}{M}} \quad (M = 24) \quad (S8)$$

The smaller the RMS or root mean square values, the closer the calibrated colour is to the target value for all 24 colour patches.

Table S-2 demonstrates the performance of the polynomial algorithms for the colorchecker, in which the second order polynomial order (#2) showed a better RMS value than that of the linear algorithm.

Table S-2. Performance of the polynomial algorithms for Munsell Colorchecker.

Algorithm #	Munsell Colorchecker RMS				Average RMS
	Incandescent	Fluorescent	LED	Daylight	
1	6.57	6.63	6.59	5.67	6.37
2	4.03	4.34	4.40	3.11	3.97

Where the algorithm orders are as follows:

#1: $x : [R, G, B, 1]$

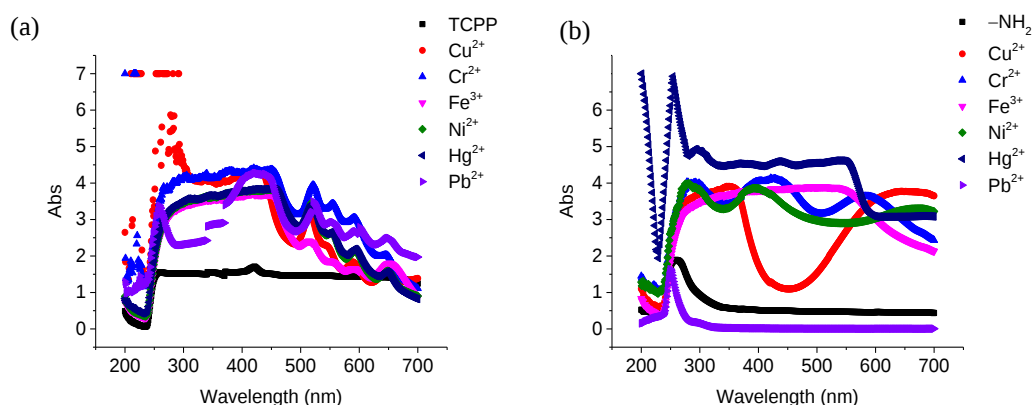
#2: $x : [R, G, B, R^2, G^2, B^2, RG, RB, GB, 1]$

The performance of the polynomial algorithms #1 and #2 was compared to evaluate their performance in determining the colour difference between the HMI images. Table S-3 demonstrates the average RMS value of Cu^{2+} ions interacting with TCPP, $-\text{NH}_2$ and MePh indicators. From this table; both algorithms are equally accurate. However, algorithm #2 was used to calibrate the HMI images because it showed the best average RMS values for both Munsell colorchecker and HMI images on the μPADs .

Table S-3. Performance of the polynomial algorithms for Cu^{2+} complex.

Algorithm #	RMS of 0.1 M Cu^{2+}			Average RMS
	TCPP	$-\text{NH}_2$	MePh	
1	5.43	5.07	3.60	4.70
2	6.87	3.25	4.04	4.72

4. METAL-LIGAND COMPLEXES ENERGY GAP

**Figure S-1.** UV-vis absorption spectrums of 0.1 M HMIs reaction with (a) TCPP and (b) $-\text{NH}_2$ indicators.

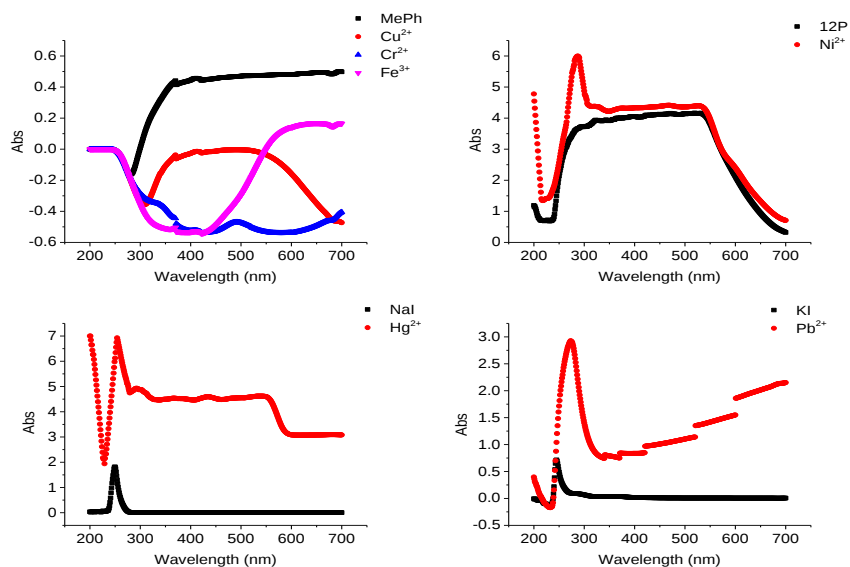


Figure S-2. UV-vis absorption spectrums of 0.1 M HMIs reaction with third indicators.

Table S-4. HOMO-LUMO energy gaps of the chelate structures of triple indicators and heavy metal ions (0.1 M) from the UV-vis measurement. The experimental HOMO-LUMO energy gaps = hc/λ_{max} , $h=6.626 \times 10^{-34} \text{ J.s}$, $c= 3 \times 10^8 \text{ m/s}$.

HOMO-LUMO energy gap (eV)				
Experimental (λ_{max}/nm)				
HMIs	TCPP (Soret Band)	TCPP (Q Bands)	–NH ₂	Third Ligand
Cu ²⁺	2.89 (428 nm)	510, 550, 595, 650	3.88 (319 nm)	-
Fe ³⁺	2.74 (451 nm)	512, 550, 595, 655	2.76 (450 nm)	2.07 (600 nm)
Ni ²⁺	2.70 (458 nm)	515, 550, 595, 650	3.22 (385 nm)	4.33 (286 nm)
Cr ²⁺	2.73 (453 nm)	515, 550, 595, 650	2.76 (450 nm)	-
Hg ²⁺	2.70 (458 nm)	515, 550, 595, 650	2.82 (440 nm)	4.86 (255 nm)
Pb ²⁺	2.93 (423 nm)	513, 550, 600, 650	4.96 (250 nm)	4.54 (273 nm)

5. HEAVY METAL IONS WASTEWATER DISCHARGE CONCENTRATIONS

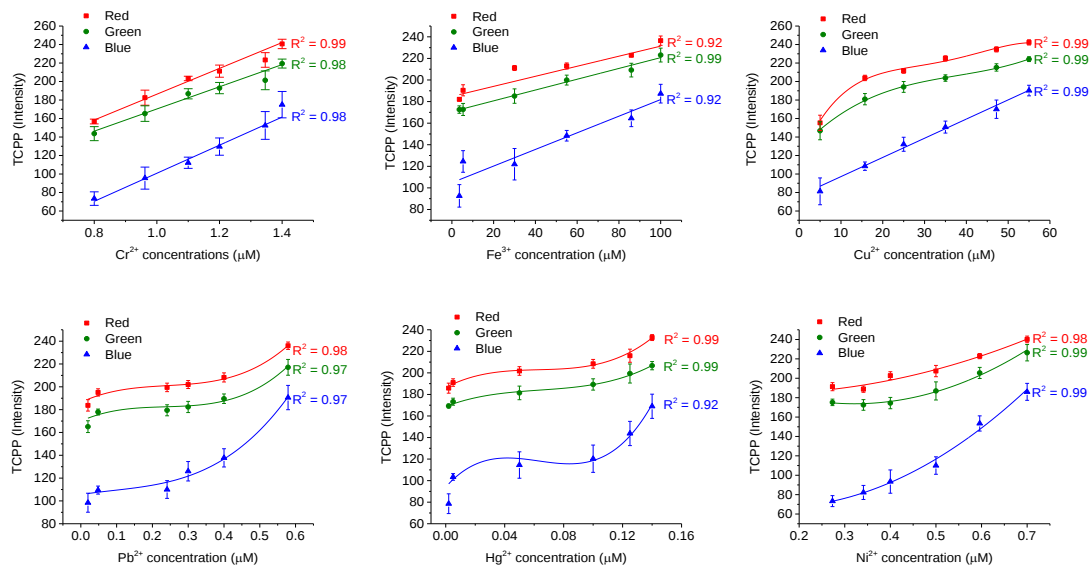


Figure S-3. RGB colour profile of the interaction of single HMIs with TCPP as a function of concentration.

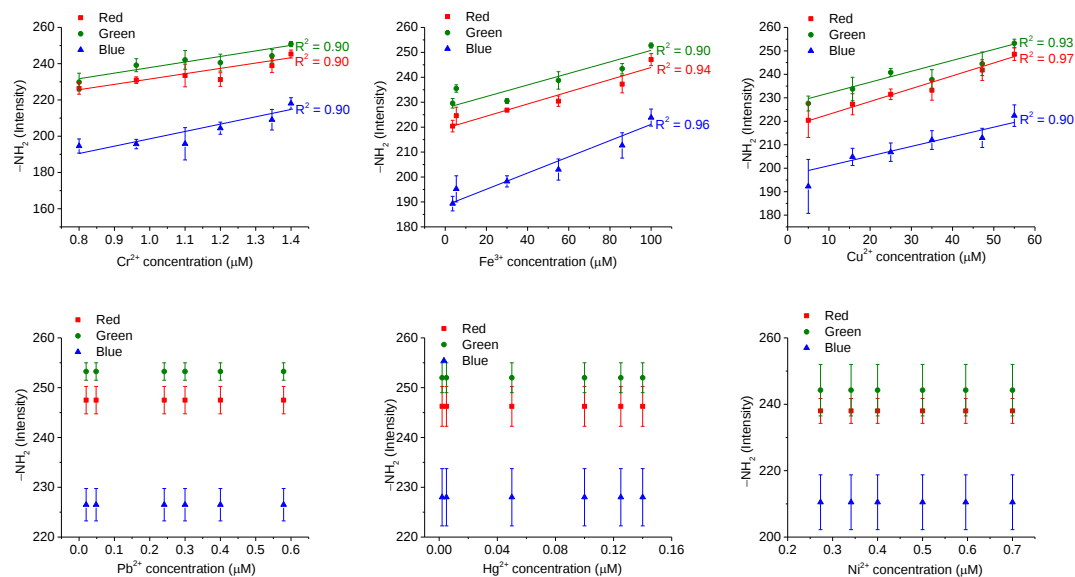


Figure S-4. RGB colour profile of the interaction of single HMIs with $-\text{NH}_2$ as a function of concentration.

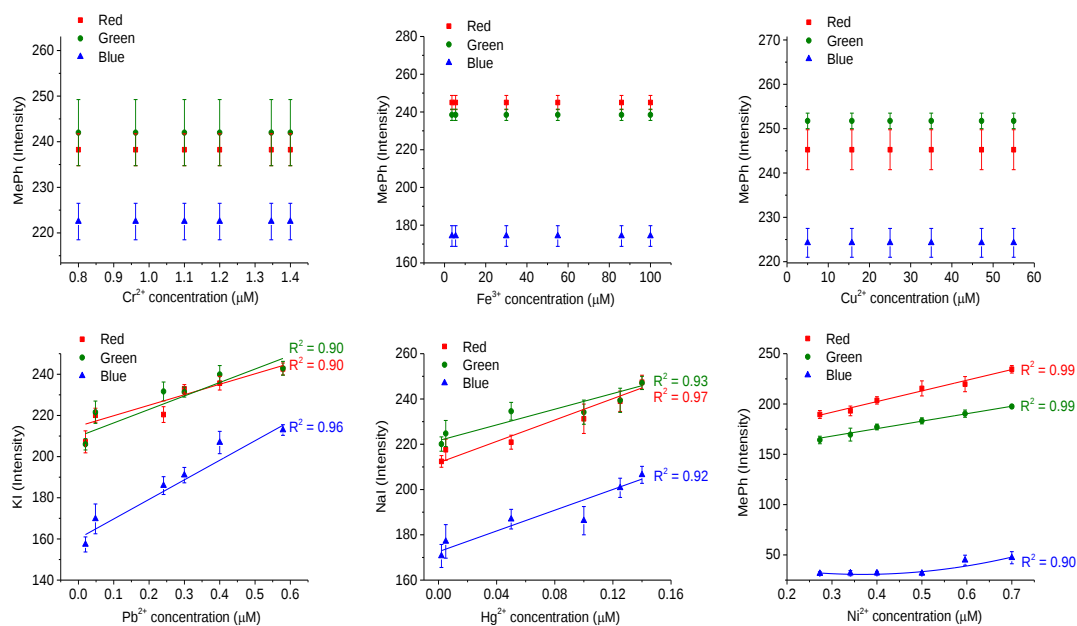


Figure S-5. RGB colour profile of single HMIs interaction with several third indicators as a function of concentration.

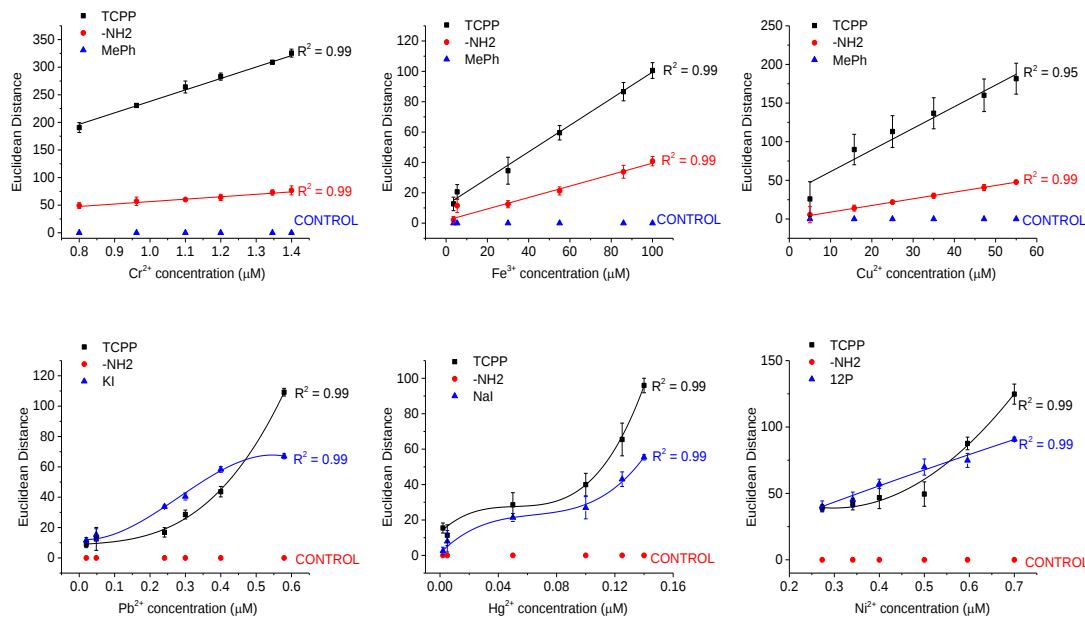


Figure S-6. Linear composition based on the Euclidean distances at wastewater discharge concentrations of single HMIs.

6. CHOICE OF PAPER AND FABRICATION OF THE MICROFLUIDIC CHANNEL

The choice of paper is very important in order to achieve better flow of the analytes into the detection zones where the ligands are located, thus ensuring uniform colour formation. In this experiment, the performance of Whatman® qualitative filter paper, Grades 1 and 4 were studied. Their performance was examined in terms of factors: (a) volume of ligands, (b) reaction colour quality between the ligand and the target analyte, and (c) the microfluidic channel resolution. Properties of both papers are listed in Table S-5.

Table S-5: Whatman® qualitative filter paper properties.

Paper properties	Grade 1	Grade 4
Pore size (particle retention)	11 µm	20-25 µm
Thickness	180 µm	205 µm
Flow speed (Herzberg)	150 sec/100 mL	37 sec/100 mL
Ash	≤ 0.06%	≤ 0.06%
Material	Cellulose filters	Cellulose filters

6.1. Volume and Colour Formation

Figure S-7 demonstrates the µPADs pattern. The pattern consists of three major areas that include: (i) target zone; onto which the target HMIs are dropped, (ii) channel; through which the HMIs flow towards (iii) the detection zone, where the ligands were preloaded. L₁, L₂, and L₃ correspond to the triple indicators/ligands. Using this pattern, the volume of ligands, and the colour quality of ligand-HMI interactions were studied.

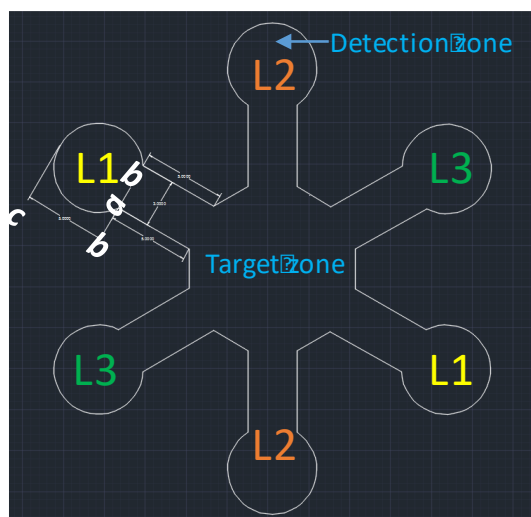


Figure S-7: μ PADs pattern: a =channel width, b =channel length, c = diameter of detection zone. Target zone diameter is approximately 10 mm.

Washburn Equation²;

$$L = \sqrt{\frac{\gamma D t}{4 \mu}} \quad (\text{S9})$$

where L is the distance moved by the fluid front, γ is the effective surface tension of the liquid, D is the average pore diameter, t is time, and μ is the viscosity of the liquid demonstrates that the time for HMIs to reach the detection zone is approximately $t \approx 4 L^2$. Doubling the channel length thus slows down the time needed for fluid to reach the detection zone by approximately four times. In addition, doubling the channel length is expected to require double the HMI volume. Thus, a suitable channel length is highly desired for rapid reactions and low-volume applications.

To obtain a suitable channel length, L , Whatman[®] qualitative filter paper Grade 1 and 4 were cut by hand to different channel lengths of 10 cm and 5 cm, using scissors. Next, several volumes of ligands, as shown in Figure S-8 and Figure S-9, were pipetted onto the detection zones. The ligands were preloaded on the detection zones of $L_1 = \text{TCPP}$, $L_2 = -\text{NH}_2$ and $L_3 = \text{MePh}$. Their fluid front was marked with a black ink, and their flow time was recorded. The aim of this experiment was to attain little volume with a fluid front covering $\frac{3}{4}$ of the channel length. For both Grade 1 and 4 with channel length $L = 10$ mm, the required volume for the ligands to reach $\frac{3}{4}$ of the length was between 2-3 μL . If $L = 5$ mm, only 1 μL was needed to achieve the same result. This experiment showed that shortening the channel length by half

meant that the ligand volume required to have a fluid front of $\frac{3}{4}$ the channel length was decreased by half. However, this result did not demonstrate the selectivity of the paper.

In order to test the selection of paper, two parameters were examined: (i) the volume of HMI required to obtain (ii) full colour formation of the metal complex with 1 μL of ligands. In this experiment, a copper solution of Cu^{2+} was used. For both paper grades with $L = 10\text{ mm}$, a HMI volume of 5 – 40 μL was not able to reach the entire detection zone, as indicated by the black marks in Figure S-10 and Figure S-11. The full colour formation between L_2 i.e., $-\text{NH}_2$ and Cu^{2+} for $L = 10\text{ mm}$ is observed to be 100 μL . If the channel length was shortened to 5 mm, a superior uniform blue Cu^{2+} complex was demonstrated on Grade 1 paper compared to the thicker Grade 4. Therefore, this experiment showed that using 1 μL of ligand, 50 μL of metal solution is required for $L = 5\text{ mm}$ on Grade 1 paper.

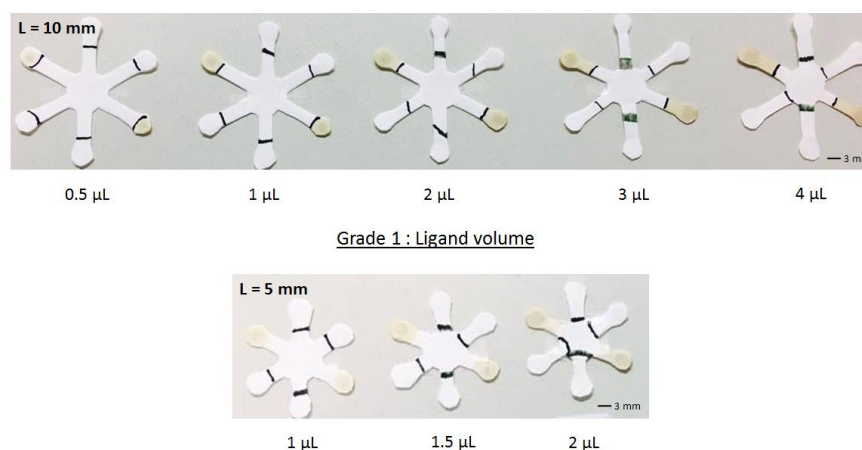


Figure S-8: Grade 1: Hand-patterned μPADs made using commercial scissors to study the ligand volume requirement for channel sizes of 10 mm and 5 mm.

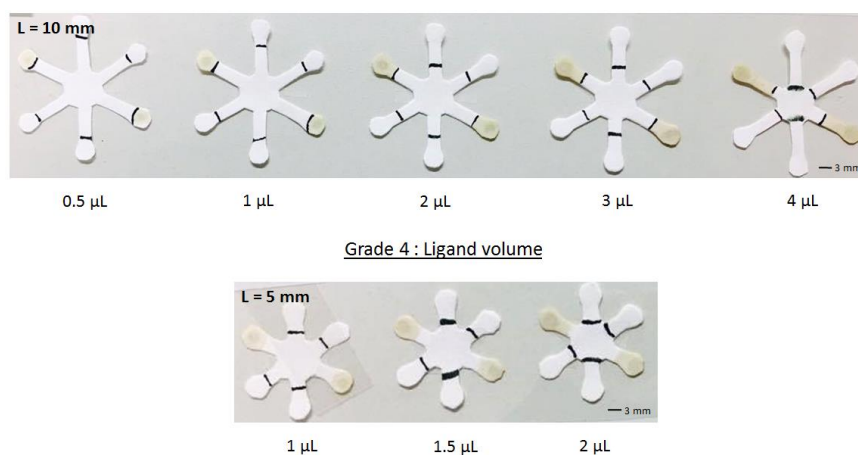


Figure S-9: Grade 4: Hand-patterned μ PADs made using commercial scissors to study the ligand volume requirement for channel sizes of 10 mm and 5 mm.

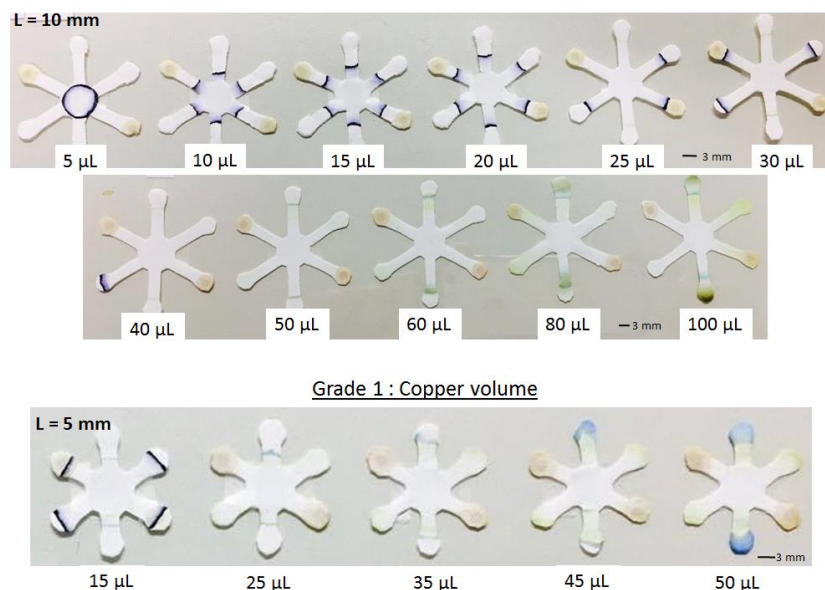


Figure S-10: Grade 1: Hand-patterned μ PADs made using commercial scissors to study the volume requirement and colour formation of Cu^{2+} HMI. For channel sizes of 10 mm and 5 mm, 3 μ L and 1 μ L of ligands were used.

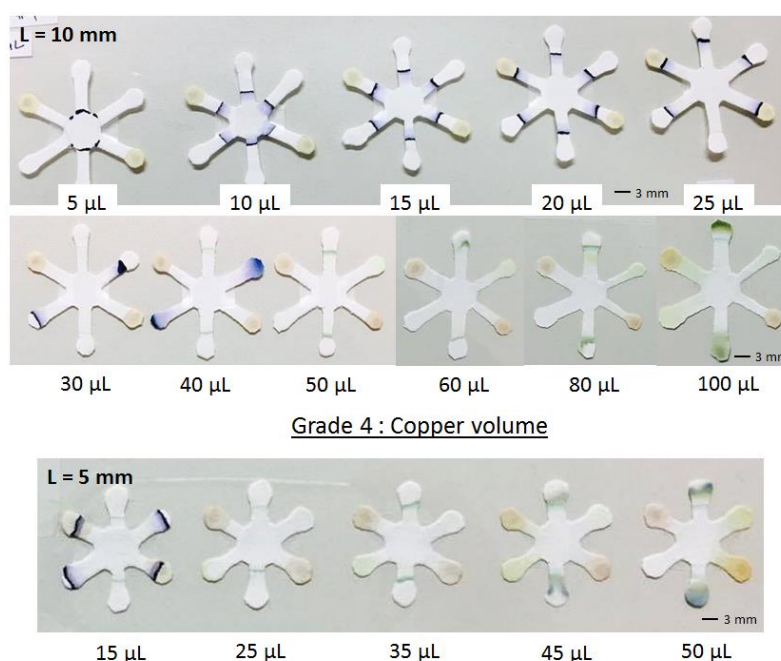


Figure S-11: Grade 4: Hand-patterned μ PADs made using commercial scissors to study the volume requirement of Cu^{2+} HMI. For channel sizes of 10 mm and 5 mm, 3 μ L and 1 μ L of

ligands were used.



(a) Grade 1: 30 seconds



(b) Grade 4: 60 seconds

Figure S-12: Reaction time of Cu^{2+} with multiple ligands: (a) Grade 1 with 30 seconds, and (b) Grade 4 with 60 seconds for each μPADs .

Further tests were conducted to confirm the reproducibility and uniform colour formation of the complexes using Grade 1 paper. Figure S-12 demonstrates 5 out of 6 reproducible coloured Cu^{2+} complexes compared to the other grade. In addition, Grade 1 paper showed a chelation time twice as fast as its counterpart.

6.2. Stamping Resolution

As shown from the previous study, Grade 1 paper possesses better properties for μPADs . In this section, the spreading of molten wax in paper was studied to determine the best stamping time for fabrication of μPADs using a metal stamp. Wax spreading is a process of capillary flow in porous materials that is described by the Washburn Equation (S9). The viscosity of the wax, μ , is a function of the temperature, and a constant and well-controlled heat source is required for reproducible results.

Assuming that the paper is kept at a constant temperature throughout the heating step, a constant surface tension of wax and average paper pore size, the distance, L , that the wax will spread in the paper is approximately, $L \approx \sqrt{\frac{t}{4}}$, where t is the stamping time. L measures the amount of wax diffused into the non-wax area after stamping, thus, decreasing the diameter of the non-wax area. A simple wax stamping process is conducted using a brass stamp to study the spreading of wax, as shown in Figure S-13.

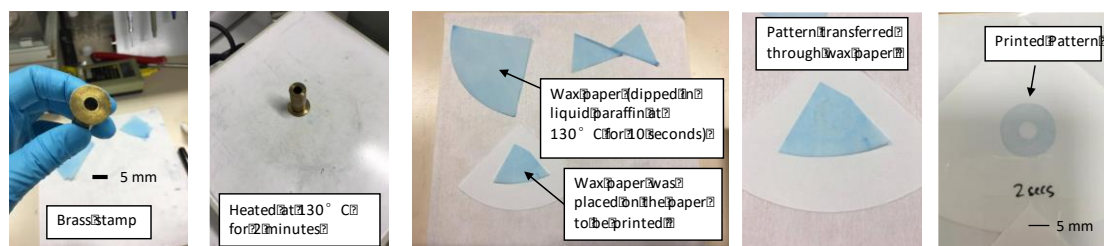


Figure S-13: Simple wax stamping process using a brass stamp.

As a proof-of-concept, commercial candles were used because they are inexpensive, easily obtained from the store, and the resolution of the transferred pattern can be easily identified by their blue color. The candles were melted on a hotplate at 130°C. Both papers of Grade 1 and 4 were dipped into the liquid paraffin for 10 seconds, or until they the papers are fully covered in wax, and then the papers were taken out and left to dry. The ‘wax paper’ was then placed on top of a fresh paper of similar grade, ready for stamping. Second, the brass stamp with an opening (hole) size of 5 mm was heated at 130°C on a hot plate for 2 minutes. After that, the brass stamp was brought into contact on the ‘wax paper’ as illustrated in Figure S-13 for a variety of time. This stamping technique is motivated by Garcia et al.³ The stamped patterns shown in the figure, experienced a leakage of water of 1 second stamping time. Stamping times of 2, 5 and 10 seconds demonstrated successful wicking of 1 μL water and a ‘ring’ appearance around their reservoirs.

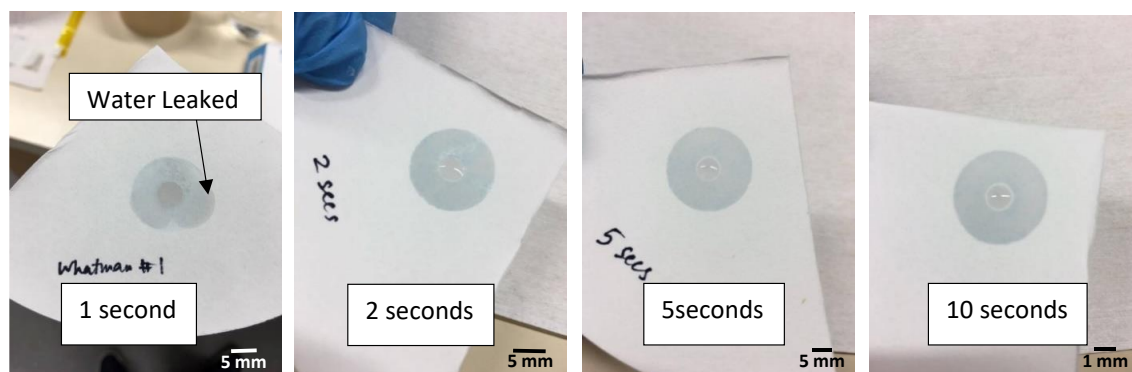


Figure S-14: Stamped pattern on Grade 1 paper for stamping time = 2, 5 and 10 seconds. The reservoir was wetted with 1 μL of deionized water.

Figure S-14 shows a ring of wax diffusion observed in the presence of a drop of water in the reservoir for stamping times of 2, 5, and 10 seconds. A schematic of the diffusion ring indicated by *X* is illustrated in Figure S-15.

An expression that correlates the width of the hydrophilic reservoir to the width of the original brass opening diameter is expressed in Equation (S10):

$$\phi = D - 2X \quad (\text{S10})$$

where ϕ is the diameter of the transferred pattern or reservoir, D is the diameter of the metal stamp, and X is the wax diffusion ring. This relationship helps to calculate the dimensions of a printed pattern required to produce a given μ PAD, as listed in Table S-6. It shows that the experimental values of the stamping resolution were close to the theoretical values.

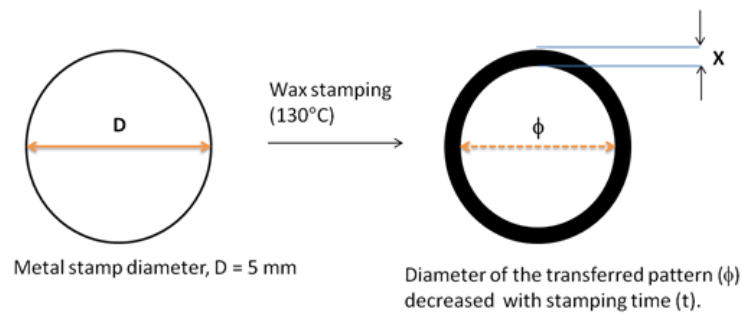


Figure S-15: The resolution or diameter change after stamping. X indicates the diffusion ring.

According to Carrilho et al.⁴ the molten wax spreads vertically, creating hydrophobic barriers across the paper thickness, and laterally into the paper decreasing the resolution of the printed pattern, resulting in hydrophobic barriers that are wider than the original printed patterns. Usually the lateral spreading of wax in paper is more rapid than vertical spreading because fibres in paper tend to be more horizontal than vertical. This explains the appearance of the wax diffusion rings.

Table S-6: Dimensions of the transferred pattern with respect to stamping time: experimental and theoretical values.

Stamping time (seconds)	D (mm)	X (mm)	$\phi_{\text{experiment}} = D - 2X$ (mm)	$L \approx \sqrt{\frac{t}{4}}$ (mm)	$\phi_{\text{theory}} = D - 2L$ (mm)
2	5	0.5	4	0.7	3.6
5		1	3	1.1	2.8
10		2	1	1.6	1.8

Although the spreading of molten wax in paper complicates the design of the μ PADs since the dimensions of the printed patterns on paper are not the same as the original, the wax stamping

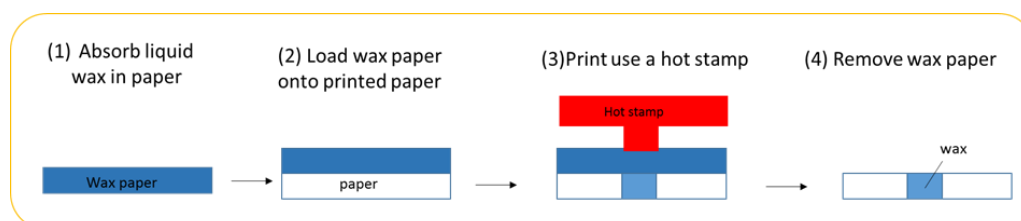
method is extremely inexpensive, and simpler than the photolithography method, and is adequate for most μ PADs applications, such as the detection of heavy metals.

The most widely used paper in sensor fabrication is Whatman Grade no. 1 chromatography paper. This is because the paper has a smooth and uniform surface on both sides, a medium flow rate, and 0.18 mm thickness that permits printing in commercial machines. It is important to highlight that the Grade 1 paper consists of 98% α -cellulose, with no additives used such as instance strengthening or whitening agents, thus reducing the possibility of interference.⁵⁻⁷

Therefore, Grade 1 paper shows the best properties and was chosen to be used in the fabrication of μ PADs using the wax stamping method.

6.3. Fabrication of the paper-based microfluidics

A handheld and lightweight stainless-steel stamp as illustrated in Figure S-16 was used to transfer the pattern for the μ PADs. To fabricate the stamp, the layout of the microfluidic structure was machined on the metal surface and then used for the rapid prototyping of μ PADs following the protocol of brass stamping and Scheme S-1.



Scheme S-1: Schematic of the wax stamping method using a metal stamp.

The advantage of this technique is that the stamp was designed to have larger protrusions in the parts of the design where hydrophobic barriers will reside. This allows for control of the wax diffusion, and thus better channel resolution.

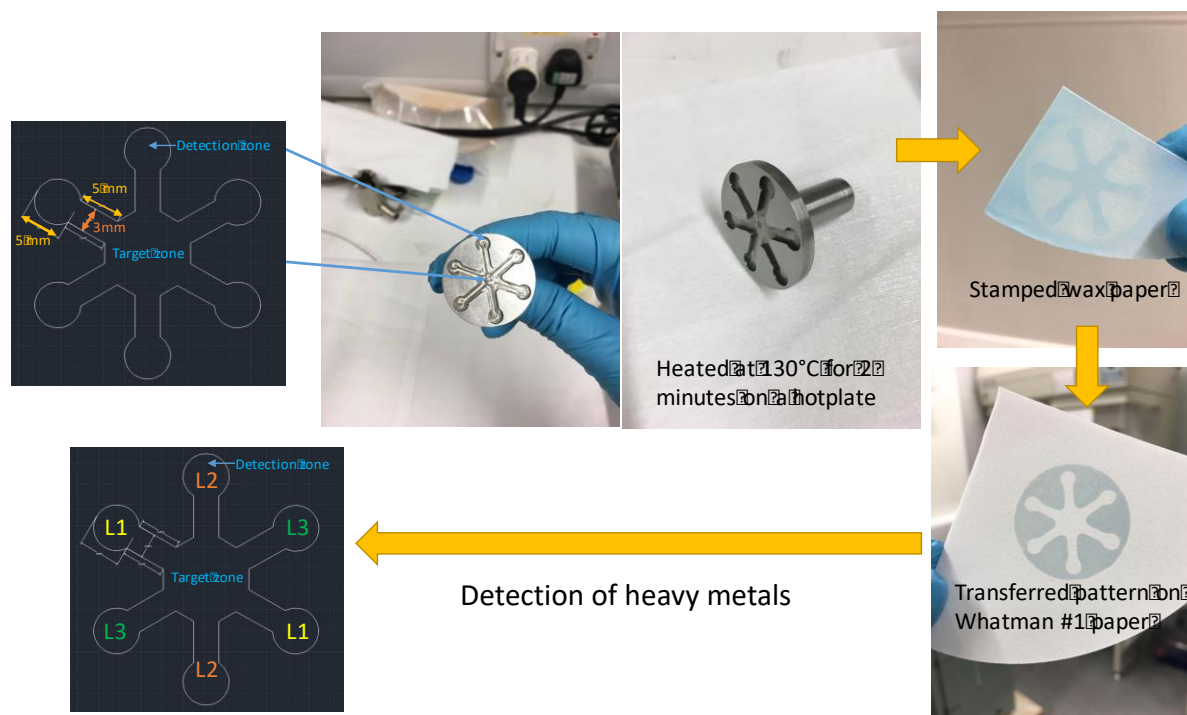


Figure S-16: Experimental process of μ PADs fabrication using a stainless-steel stamp. L1, L2 and L3 correspond to the multiple ligands.

Once the μ PADs are fabricated, the indicators/ligands containing ions or molecules are preloaded on the detection zone of the μ PADs and subsequently covered with a black paper, since the TCPP porphyrin ligand is light sensitive. This is done as follows, firstly, a standard 80 gsm black paper with a hole carved in the centre, is mounted on an acetate sheet, using a cellotape, as illustrated in Figure S-17. The hole in the centre of the black paper (Figure S-17) was superimposed on the target zone to allow for loading the metal ion solution. Next, the acetate sheet with the black paper was placed on top of the μ PAD, with the black sheet covering the detection zones of the μ PAD, to prevent exposure to visible light. Finally, the black paper is lifted 30 seconds after the addition of a metal ion solution, and the color changes are computed using digital imaging and color correction algorithm.

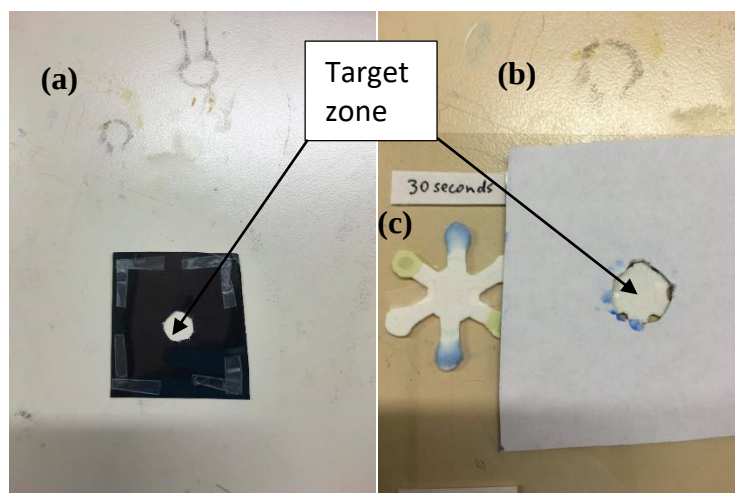


Figure S-17: Preparation of sensing platform: (a) the black paper mounted on an acetate sheet with a hole carved in the centre, (b) after loading the ligands on the detection zones of the μ PAD, the μ PAD is covered with the black paper and (c) the black paper is lifted 30 seconds after the addition of metal ion solution, Cu^{2+} on the target zone.

7. DISCRIMINATORY CAPACITY

To further evaluate the capability of this multidimensional sensor and discrimination of single heavy metal ions based on interaction with multiple indicators, a clustering of the linear composition responses was carried out using MATLAB hierarchical clustering. It is a model-free method based on grouping the heavy metal ion vectors per their spatial distances in their full multidimensional arrays. All six single ions are accurately classified, illustrated by the dendrogram in Figure S-18 that shows strong sensor discriminatory capacity.

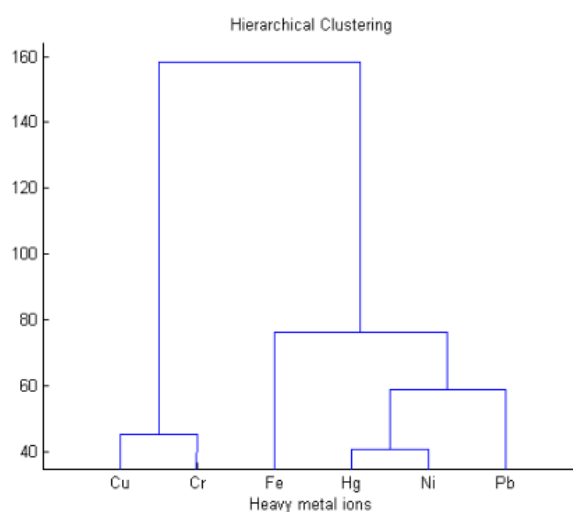


Figure S-18. Dendrogram formed by MATLAB hierarchical clustering of the sensor response.

8. STUDY OF ION COMPOSITIONS IN A MIXTURE

The mixing ratio of (Cu, Cr, Fe) are $a = (0, 1/3, 2/3)$, $b = (0, 2/3, 1/3)$, $c = (1/3, 0, 2/3)$, $d = (2/3, 0, 1/3)$, $e = (2/3, 1/3, 0)$, $f = (1/3, 2/3, 0)$, and $g = (1/3, 1/3, 1/3)$. These ratios are taken from 0.1 M concentrations of each ion. Average RGB values in a mixed composition was used to calculate the coefficient coordinates by means of least-squares solution. The relationship coefficients α , β , and γ shown in Equation (S11) and Figure S-19 correspond to the presence of Cu, Cr, and Fe.

$$\begin{pmatrix} r_{mix} \\ g_{mix} \\ b_{mix} \end{pmatrix} = \alpha \begin{pmatrix} r_{Cu} \\ g_{Cu} \\ b_{Cu} \end{pmatrix} + \beta \begin{pmatrix} r_{Cr} \\ g_{Cr} \\ b_{Cr} \end{pmatrix} + \gamma \begin{pmatrix} r_{Fe} \\ g_{Fe} \\ b_{Fe} \end{pmatrix} \quad (S11)$$

Euclidean distances (EDs) calculate the difference of α , β , and γ between mixture compositions and non-mixed ions of Cu, Cr and Fe. (1, 0, 0), (0, 1, 0) and (0, 0, 1) coordinates at 0.1 M correspond to 100% presence of Cu, Cr and Fe, respectively. The presence of ions in a mixture can be identified based on their interaction with multiple indicators for each mixing concentration ratios. Using multiple indicator strategy, it is anticipated that each ion can be identified and tabulated in Table S-7 and S-8.

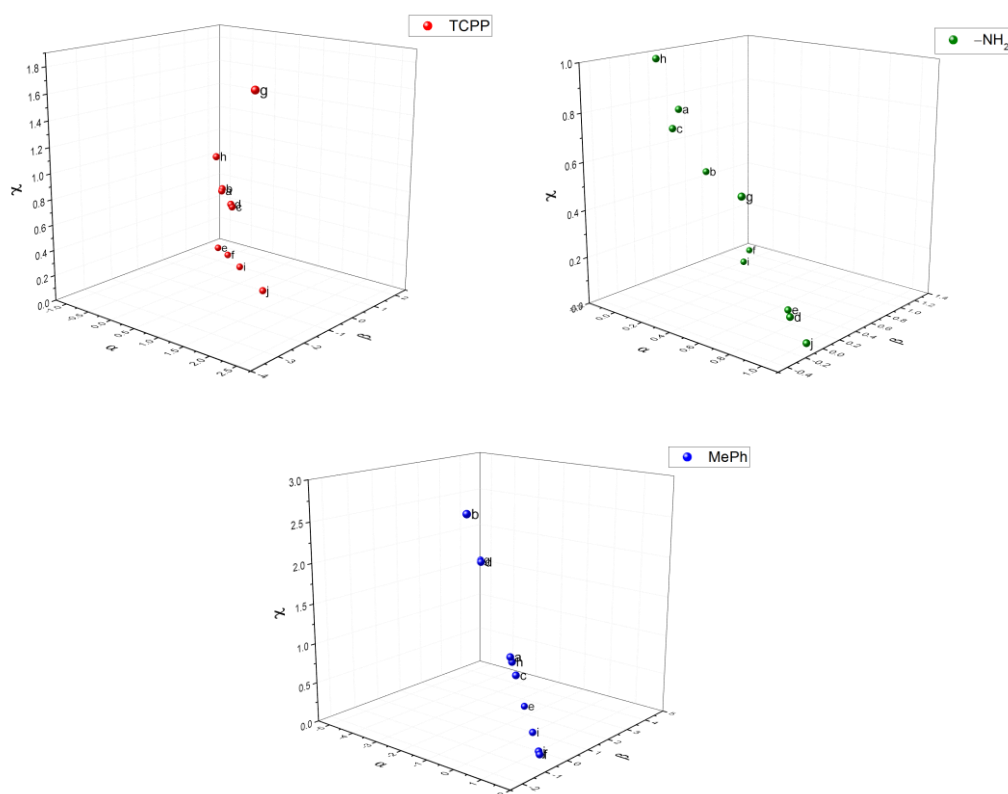


Figure S-19: The coefficient coordinates in terms of α , β , and γ that correspond to the presence of Cu^{2+} , Cr^{2+} and Fe^{3+} at different ratios.

Table S-8 demonstrates that high concentrations of Fe^{3+} in mixture ratio a and c, resulted in only Fe determination. This may be due to stronger absorption of visible light by Fe^{3+} (see Figure S-1 and S-2, and Table S-4) thus, dominating the resultant color of the ‘mixed’ complexes. If the concentration of Cu^{2+} and Cr^{2+} are twofold than the concentration of Fe, the individual composition of Cu^{2+} and Cr^{2+} are traceable; in the case of mixture b and d. For mixture e and f, both Cu^{2+} and Cr^{2+} are detected for different concentration ratios. With equal concentration of Cu^{2+} , Cr^{2+} and Fe^{3+} in mixture g, all of the ions were successfully detected.

Table S-7: Euclidean distance between the mixed and the individual (non-mixed) ions.

TCPP				Euclidean Distance																			
	α	β	γ		h	i	j		h				i				j				Closes to		
a	0	0.22	0.71		0.37	1.05	1.24		0	0	1		0	1	0		1	0	0		h		
b	0	0.25	0.72		0.37	1.05	1.26		0	0	1		0	1	0		1	0	0		h		
c	0.34	0	0.62		0.51	1.23	0.91		0	0	1		0	1	0		1	0	0		h		
d	0.31	0	0.64		0.48	1.23	0.94		0	0	1		0	1	0		1	0	0		h		
e	-0.93	1.83	0		2.28	1.25	2.66		0	0	1		0	1	0		1	0	0		i		
f	-0.55	1.54	0		1.92	0.78	2.19		0	0	1		0	1	0		1	0	0		i		
g	2.46	-3.34	1.83		4.23	5.31	4.08		0	0	1		0	1	0		1	0	0		j		
NH2				Euclidean Distance																			
	α	β	γ		h	i	j		h				i				j				Closes to		
a	0	0.23	0.78		0.32	1.09	1.29		0	0	1		0	1	0		1	0	0		h		
b	0	0.54	0.48		0.75	0.66	1.23		0	0	1		0	1	0		1	0	0		i		
c	0.11	0	0.73		0.29	1.24	1.15		0	0	1		0	1	0		1	0	0		h		
d	0.9	0	0.08		1.28	1.35	0.13		0	0	1		0	1	0		1	0	0		j		
e	0.67	0.4	0		1.27	0.9	0.52		0	0	1		0	1	0		1	0	0		j		
f	-0.11	1.26	0		1.61	0.28	1.68		0	0	1		0	1	0		1	0	0		i		
g	0.75	-0.29	0.58		0.91	1.6	0.69		0	0	1		0	1	0		1	0	0		j		
MePh				Euclidean Distance																			
	α	β	γ		h	i	j		h				i				j				Closes to		
a	0	-0.08	1.07		0.11	1.52	1.47		0	0	1		0	1	0		1	0	0		h		
b	0	-1.92	2.85		2.66	4.08	3.58		0	0	1		0	1	0		1	0	0		h		
c	0.15	0	0.85		0.21	1.32	1.21		0	0	1		0	1	0		1	0	0		h		
d	-1.16	0	2.11		1.61	2.61	3.02		0	0	1		0	1	0		1	0	0		h		
e	-1.53	2.54	0		3.12	2.16	3.57		0	0	1		0	1	0		1	0	0		i		
f	1.16	-0.16	0		1.54	1.64	0.22		0	0	1		0	1	0		1	0	0		j		
g	-4.92	4.35	1.58		6.59	6.16	7.51		0	0	1		0	1	0		1	0	0		i		

Table S-8: The identification of ions composition based on different indicators interaction with the mixture solution.

	(Cu ²⁺ , Cr ²⁺ , Fe ³⁺) concentration ratios	TCP	-NH ₂	MePh
a	(0, 1/3, 2/3)	(0, 0, 1)	(0, 0, 1)	(0, 0, 1)
b	(0, 2/3, 1/3)	(0, 0, 1)	(0, 1, 0)	(0, 0, 1)
c	(1/3, 0, 2/3)	(0, 0, 1)	(0, 0, 1)	(0, 0, 1)
d	(2/3, 0, 1/3)	(0, 0, 1)	(1, 0, 0)	(0, 0, 1)
e	(2/3, 1/3, 0)	(0, 1, 0)	(1, 0, 0)	(0, 1, 0)
f	(1/3, 2/3, 0)	(0, 1, 0)	(0, 1, 0)	(1, 0, 0)
g	(1/3, 1/3, 1/3)	(1, 0, 0) & (0, 0, 1)	(1, 0, 0)	(0, 1, 0) & (0, 0, 1)

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